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REGENERATION OF *PLEUROTRICHA* AFTER MEROTOMY WITH REFERENCE ESPECIALLY TO THE NUMBER OF MICRONUCLEI AND THE OCCURRENCE OF UNINUCLEATE CELLS.[1]

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Lewin,[1] working with *Stylonychia*, has reported a series of experiments in which, after merotomy, he observed an increase in the number of micronuclei in the regenerated merozoa. It was thought to be of interest to repeat Lewin's experiments on another member of the hypotrichus group to determine if there might be any general application of the phenomenon he had observed. Pleurotricha was chosen for the experiments.

MATERIAL.

The animals used were secured from a strain, the originator of which was isolated by Dr. George A. Baitsell in the biological laboratory of Yale University. The strain had been adapted to laboratory hay infusion media and was preserved as a stock culture in sterile test-tubes plugged with cotton. Such a culture tube of organisms was given me and from it subcultures were made to other tubes of fresh media and to fresh media in small glass capsules.

The hay infusion used as medium was prepared by placing about 10 grams of field hay in 200 c.c. of tap water in an Erlenmeyer flask and boiling it over a bunsen burner for a few minutes. This medium was made up but once and was used in the proportion of one drop of the infusion to five drops of tap water, this amount of fluid in a capsule serving as the medium for a single animal for twenty-four hours, or till it divided.

[1] From the Marine Biological Laboratory, Woods Hole, Mass.

Method.

Merotomy was performed according to the method of Calkins. The animal selected was drawn up into a fine pointed pipet and placed on a clean glass slide under a Greenough's binocular microscope, eye-pieces 4 and objectives a. The medium was then drawn off till a drop of only sufficient size for the animal to swim in freely was left. With an ophthalmologist's iridectomy knife the animals were cut in parts. The point was ground off the knife and one edge ground to a semi-bellied shape. The blade was plunged into the drop with the animal and the posterior point of its edge allowed to rest on the surface of the slide. As the animal passed from one side to the other of the drop, or around the resting point of the knife edge, successive attempts were made by moving the knife handle up and down to cut the animal as it came directly in line with the edge of the knife.

The frangibility of the cell body afforded one of the first methods of securing fragments of infusoria for study. *Pleurotricha* appears to be very frangible. On one occasion, experiment 48, catching an animal on the surface of the media with a sudden and forcible blast of air from a fine pointed pipet the animal was broken in two. Simply drawing the animal rather forcibly in the pipet was sometimes sufficient to break it in two as in experiment 40. In a few of the experiments the merozoa were secured by drawing the animal selected into a fine pointed pipet and spurting it out forcibly on the side of the capsule.

Animals to be stained were isolated from the capsule with a fine pipet on a clean microscopical slide under a binocular microscope. They were killed and fixed in 5 per cent. glacial acetic acid in saturated mercuric chloride, stained by the Heidenhain iron hematoxylin method, and mounted in xylol balsam.

The Effect of Merotomy on the Number of Micronuclei.

In Tables I. to V., inclusive, are recorded 27 experiments in which the regenerated merozoön was recovered, successfully stained, and mounted. The tables are divided according to the position of the cut. They also state the exact time before or after division, when it had been observed, the length of time after merotomy before the animal was killed, and the number of macronuclei and micronuclei found in the stained merozoön.

In experiments 31, 32, 33 and 57 the animals were killed from 38 minutes to 3 hours and 30 minutes after merotomy. All of these, except experiment 31, were mid-body cuts and it is reasonable to assume that the condition found is normal,—the macronucleus and the micronucleus having not yet divided, or being cells of the uninucleate variety which were also found in control

TABLE I.

ANTERIOR-END CUTS.

Exp. No.	When Cut.	When Killed.	Number of Macronuclei, Number of Micronuclei.
45	—	9 hrs. 53 min. after cut.	2 macronuclei, 2 micronuclei
105	Before division	27′ 25″ after cut.	2 macronuclei, 2 micronuclei

cultures of this animal. In experiment 57 the micronucleus is in mitosis and the cell appears normal.

In experiment 22, also a mid-body cut, 1 hour and 10 minutes after operation, there are two micronuclei in mitosis. The nucleus is single, swollen and enlarged, as if ready to divide. The most plausible explanation of the condition found here is that the cell originally had two macronuclei and three micronuclei, as was found in certain animals taken from culture.

TABLE II.

POSTERIOR-END CUTS.

Exp. No.	When Cut.	When Killed.	Number of Macronuclei, Number of Micronuclei.
4	1 hr. 7 min. after division.	3 hrs. 18 min. after cut	2 macronuclei, 2 micronuclei.
15	Before division	6 min. after cut	2 " 2 "
27	"	30 " " "	2 " 2 "
33	"	38 " " "	1 " 1 "
34	"	1 hr. 10 min. after cut	2 " 9 "
39	"	8 hrs. 27 " " "	2 " 2 "
49	"	18 hrs. 7 " " "	2 " 2 "
106	"	27 hrs. 15 " " "	2 " 2 "
107	"	26 hrs. 55 " " "	2 " 2 "
109	"	22 hrs. 36 " " "	2 " 2 "

When merotomy was performed the animal was separated in two merozoa, one of which had a single macronucleus and a single micronucleus; the other merozoön which was saved and which regenerated had a single macronucleus and two micronuclei.

In experiment 34, a posterior-end cut, 1 hour and 10 minutes after operation, we have an abnormal and an interesting animal. The cell is pointed at both ends and broad in the middle, measuring 112 x 45 micra. The anterior macronucleus is situated

TABLE III.

MID-BODY CUTS.

Exp. No.	When Cut.	When Killed.	Number of Macronuclei, Number of Micronuclei.
7	1 hr. 17 min. after division.	3 hrs. 15 min. after cut.	2 macronuclei, 2 micronuclei.
22	Before division	1 hr. 10 min. " "	1 macronucleus, 2 "
25	" "	2 hrs. 25 " " "	2 macronuclei, 2 "
31	" "	2 " 30 " " "	1 macronucleus, 1 micronucleus.
32	" "	3 " 30 " " "	1 " 1 "
35	" "	11 " 40 " " "	2 macronuclei, 2 micronuclei.
47	6 hrs. 8 min. after division.	14 " 47 " " "	2 " 2 "
51	6 hrs. 3 min. after division.	13 " 3 " " "	2 " 2 "
57	———	1 hr. 35 " " "	1 macronucleus, 1 micronucleus. (dividing)
88	Before division.	14 hrs. 3 " " "	4 macronuclei, 4 micronuclei.
93	" "	41 " 30 " " "	2 " 2 "
95	" "	45 " 21 " " "	2 " 2 "

nearer the anterior end than normally. It is round in shape, measuring 5 micra in diameter. Its chromatin is homogeneous, deeply stained, showing no vesicles or granules. The posterior macronucleus is likewise situated nearer the anterior end than normally. It measures 7 x 5 micra and its chromatin is of the

TABLE IV.

ANTERIOR- AND POSTERIOR-END CUTS.

Exp. No.	When Cut.	When Killed.	Number of Macronuclei, Number of Micronuclei.
99	Before division.	43 hrs. 47 min. after cut.	2 macronuclei, 2 micronuclei.

same character as that of the anterior macronucleus. There is one micronucleus adjacent to each macronucleus and seven other micronuclei scattered irregularly throughout the anterior portion of the cell. They are all of about the same size, 2 micra in diameter, deeply stained and homogeneous. The cytoplasm

is finely granular. The changes here appear more like those of a degenerative than those of a physiological process and may have been present at the time the animal was operated upon.

In the twenty other experiments there were two macronuclei

TABLE V.

LONGITUDINAL CUTS.

Exp. No.	When Cut.	When Killed.	Number of Macronuclei, Number of Micronuclei.
			(dividing)
86	Before division.	20 hrs. 20 min. after cut.	4 macronuclei, 4 micronuclei.
110	" "	25 hrs. 50 min. after cut.	2 macronuclei, 2 micronuclei.

and two micronuclei, the number normally and most constantly found in this strain of animals.

In Table VI. are recorded the observations made on twelve animals, descendants of merozoa and selected from various generations from the second to the thirty-fifth. In all of these animals there were two macronuclei and two micronuclei.

TABLE VI.

Exp. No.	Cut.	Generation after Cutting.	Number of Macronuclei, Number of Micronuclei.
16	Anterior.	35th	2 macronuclei, 2 micronuclei.
21	Posterior.	30th	2 " 2 "
25	Mid-body.	24th	2 " 2 "
37	Anterior.	10th	2 " 2 "
53	"	2d	2 " 2 "
61	Mid-body.	4th	2 " 2 "
64	Posterior.	2d	2 " 2 "
64	"	3d	2 " 2 "
70	"	2d	2 " 2 "
70	"	3d	2 " 2 "
85	Anterior.	2d	2 " 2 "
106	Posterior.	2d	2 " 2 "

Thus, the evidence furnished by these experiments is that merotomy does not produce any change in the normal number of macronuclei and micronuclei, either in the merozoön or in its descendants as far as the thirty-fifth generation. The occurrence of cells with more micronuclei than macronuclei are more readily explained as having been mechanically produced by the operation itself or as the manifestation of an abnormal cell process that may have existed before merotomy was performed, as such cells are found in normal laboratory cultures of this animal.

The Occurrence of Animals Containing but One Macronucleus.

On four occasions animals from the stock cultures and from the cultures of the control strains when killed and stained were found to possess but one macronucleus and sometimes one and sometimes two micronuclei. These animals were all large slow swimmers or crawlers, and were selected because experience had taught that these were the animals that showed early stages of division, which were being sought at that time.

Among the descendants of merozoön c, experiment 24, a uninucleate animal with two micronuclei was found. The original merozoön was from an animal cut during division. From this it was thought that perhaps injury to the cell during division might be the cause of this occurrence, but out of a large number of animals out of this strain and other strains derived from animals cut during division no other animals with a single macronucleus were found.

In experiment 76 a large slow crawler, evidently near division, was cut anteriorly at 12:25 P.M., August 9. The anterior merozoön disintegrated immediately, the posterior merozoön increased its activity. It was isolated into a clean glass capsule with five drops of tap water and one drop of hay infusion. On August 10, 10:15 A.M., there was found in the capsule three animals, two small and of the same size and a single large animal. The interpretation made of this was that the merozoön had regenerated, divided once into two individuals and that one of the latter had again divided while the other was now approaching division. On killing and staining all three of these animals it was found that each of the small animals had two macronuclei and two micronuclei, but the large animal had only one macronucleus and two micronuclei. The macronucleus appeared about to divide.

Discussion.

Since uninucleate forms occur both among the stock cultures, the control strains and among the merozoa, a conservative inference must be that they are normal variations of this animal, possibly brought about by its being adapted to laboratory media. The relation between the two may be somewhat analogous to

that which Calkins[2] has found to exist between the so-called *Paramecium caudatum* and *Paramecium aurelia*, except that there it is a variation in the number of micronuclei; here, a variation in the number of macronuclei. It appears possible that by proper selection a strain of Pleurotricha may be obtained in which all of the animals, for a period, may show only one macronucleus.

From the experiments it appears fairly conclusive that merotomy generally has no effect on the normal number of micronuclei. Animals with less macronuclei than the normal may be found both in laboratory cultures of this animal and among the descendants of merozoa. Animals with more than the normal number of micronuclei may occur in laboratory cultures. The single instance in which there was found among the merozoa an animal with two abnormal macronuclei and several micronuclei is undoubtedly that of a degenerative or a necrobiotic cell. This is somewhat suggestive that multiple micronuclei may in general be one of the manifestations of a degenerative or a necrobiotic process in the cell.

It is to be noted that these observations are not in accord with Lewin's findings for *Stylonychia*. It appears from his paper that he had some difficulty in getting his animals to survive in the media used and another possible explanation for his observations is that they were more the results of necrobiosis and degeneration in the culture strains than the effect of merotomy.

Conclusions.

1. Merotomy has no effect other than the effect that may be mechanically produced by the operation itself on the number of micronuclei of *Pleurotricha*.

2. Animals with more than the normal number of micronuclei and less than the normal number of macronuclei may occur in laboratory strains of this animal as well as among merozoa.

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